B16

70. (New) The method of claim 38, wherein the CD8+CD28- Ts cells do not express the BY55 marker.

REMARKS

Claims 38-40 and 40 are pending in the subject application. Claim 38 has been amended to more particularly point out and distinctively claim the subject matter which the applicants regard as the invention. Support for the amendment to claim 38 can be found in the specification at page 41, line 32 through page 42, line 15 and page 146, lines 16-29. Support for the amendment to claim 39 can be found in the specification at page 42, lines 6-15 and page 146, lines 16-29. New claim 70 was added to encompass certain embodiments of claim 38. Support for new claim 70 can be found in the specification at page 42, lines 6-15 and page 164, line 26 through page 165, line 8. Applicants maintain that these amendments raise no issue of new matter. Applicants also maintain that the addition of new claim 70 does not raise an issue of new matter. A marked-up version of the amended claims is attached hereto as **Exhibit C** pursuant to the requirements of 37 C.F.R. §1.121. Accordingly, claims 38-40, 42 and 70 will be pending and under examination upon entry of this Amendment.

In view of the remarks below, applicants maintain that the Examiner's rejections have been overcome, and respectfully request that they be withdrawn.

Rejections Under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 38-40 and 42 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject

matter which the applicant regards as the invention.

Specifically, the Examiner alleged that claim 38 is indefinite and ambiguous in the recitation of a) contacting the APC with Ts and b) overexpressing mRNA in the APC because "[i]t is unclear if mRNA will be expressed in the same APC that were contacting with Ts or if it would be a different APC."

In response, without conceding the correctness of the Examiner's position, and in order to expedite prosecution, applicants have amended claim 38. Claim 38 now recites, in part, "a) contacting an APC with a *CD8+CD28-* Ts; and b) causing overexpression, *in the APC of step (a)*, of mRNA which encodes an inhibitory monocyte inhibitory receptor (MIR), thereby generating a tolerogenic antigen presenting cell (APC)." (emphasis added).

In view of the above remarks, applicants maintain that amended claim 38, and claims 39, 40 and 42 satisfy the requirements of 35 U.S.C. §112, second paragraph, and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Rejections Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 38-40 and 42 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner states that while being enabling for a method of generating a tolerogenic antigen presenting cell which comprises: a) contacting the

APC with Ts, wherein Ts are CD8+CD28- T cells and b) overexpressing mRNA which encodes a MIR in the said APC, wherein the MIR is selected from the group recited in claim 39, the specification is not enabling for a method of generating a tolerogenic antigen presenting cell which comprises: a) contacting the APC with *any* Ts, and b) overexpressing mRNA which encodes *any* MIR in the said APC.

In response, without conceding the correctness of the Examiner's position, and in order to expedite prosecution, applicants have amended claim 38. Claim 38 now recites, in part, "a) contacting an APC with a *CD8+CD28-* Ts; and b) causing overexpression, in the APC of step (a), of mRNA which encodes an *inhibitory* monocyte inhibitory receptor (MIR), thereby generating a tolerogenic antigen presenting cell (APC)." (emphasis added).

Applicants contend that the specification provides support for the method of claim 38 wherein an *inhibitory* monocyte inhibitory receptor is overexpressed. Applicants direct the Examiner's attention to page 146 lines 13-24 of the specification which discloses, in relevant part, that ILT4(MIR10), ILT2(MIR) and ILT3 are upregulated in anergic APCs.

Applicants note that it was known in the art at the time of the invention that there are two subsets of monocyte inhibitory receptors. Colonna et al., on page 375, second column, last paragraph through page 376, first column, first paragraph, teach that one subset of monocyte inhibitory receptors (ILT2, ILT3, ILT4, ILT5 and LIR8) mediate inhibition of cell activation and another subset of receptors (ILT1, ILT1-like protein, ILT7, ILI8, and LIR6) activate cells (Colonna et al., 1999; attached hereto as **Exhibit D**).

Applicants note that the genus "inhibitory monocyte inhibitory receptors" comprises five species. Applicants again note support in the specification for *inhibitory* monocyte inhibitory receptors. Indeed, within the specification, explicit recitation of ILT2, ILT3 and ILT4 can be found on page 146, lines 16-21.

M.P.E.P. §2163 states that "[t]he written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species ... A representative number of species means that the species which are adequately described are representative of the entire genus."

Again, applicants stress that three species out of the "*inhibitory* monocyte inhibitory receptors" genus of five species - *over half* - are explicitly recited in the specification. Applicants maintain that such explicit description constitutes a sufficient description of a representative number of species, and thus satisfies the written description requirement for the claimed genus of *inhibitory* monocyte inhibitory receptors.

In view of the above remarks, applicants maintain that amended claim 38, and claims 39, 40 and 42 satisfy the requirements of 35 U.S.C. §112, first paragraph, and respectfully request that the Examiner reconsider and withdraw this ground of rejection.

Formalities

Drawings

In the Notice of Draftsperson's Patent Drawing Review issued

concurrently with the March 11, 2003 Office Action, the Draftsperson made certain objections to the drawings submitted in connection with the subject application.

In response, applicants attach hereto, as **Exhibit E**, 73 sheets of new, corrected formal drawings for Figures 1-37.

Sequence Listing

The Examiner stated that the specification is objected to under 37 C.F.R. §1.821(d) for failing to disclose a SEQ ID NO for the amino acid sequence disclosed on page 112, line 21.

In response, applicants submit a paper copy of a substitute Sequence Listing (**Exhibit A**) to replace the Sequence Listing filed previously filed by applicants on May 10, 2002. Applicants also submit the substitute Sequence Listing in computer-readable form on the enclosed computer diskette. Moreover, applicants submit as **Exhibit F** a Statement In Accordance With 37 C.F.R. §1.821(f) certifying that the contents of the computer-readable form and paper copy are identical, and introduce no new matter.

Applicants verify that this submission does not involve any issue of new matter. Thus, the subject application is in compliance with 37 C.F.R. §1.821-§1.825. Applicants note that the specification has been amended in order to incorporate SEQ ID NO.:382. A marked-up version of the amended paragraphs is annexed hereto as **Exhibit B**.

Specification Spelling Errors

The Examiner requested that applicants correct any errors of

which applicants may become aware in the specification. In particular, the Examiner states that "on page 5, line 25 and on page 131, line 2 the word 'Ts' is misspelled.

In response, applicants notes that on page 5, line 25 and on page 131, line 2 it is the word "Th" that is misspelled not the word "Ts". Applicants also note that these spelling errors, as well as additional spelling errors found in the specification by applicants have been corrected as discussed hereinabove.

Second Information Disclosure Statement

Applicants submit herewith a second Information Disclosure Statement under 37 C.F.R. §1.56. The references disclosed herein were originally submitted for the Examiner's consideration on August 9, 2002, but were not considered by the Examiner. Accordingly, applicants are resubmitting these references for consideration. Therefore, in accordance with their duty of disclosure under 37 C.F.R. §1.56 and 37 C.F.R. §1.97, applicants would like to direct the Examiner's attention to the following publications. Copies of cited publications are attached hereto as **Exhibits 1-9**, respectively.

1. Batliwalla, F., et al. (1996) "Oligoclonality of CD8+ T cells in health and disease: aging, infection, or immune regulation?". *Hum Immunol.* 48:68-76. Review. (**Exhibit 1**).

2. Benichou, G. and E.V. Fedoseyeva. (1996) "The contribution of peptides to T cell allorecognition and allograft rejection". *Intern. Rev. Immunol.* 13:231-243. (**Exhibit 2**).

3. Cella, M., et al. (1997) "A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing". *J. Exp. Med.* 185:1743-1751. (**Exhibit 3**).

4. Colonna, M., et al. (1998) "Cutting edge: human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules". *J. Immunol.* 160:3096-3100. (**Exhibit 4**).

5. Colovai, A.I., et al. (2000) "Induction of xenoreactive CD4+ T-cell anergy by suppressor CD8+CD28- T cells". *Transplantation.* 69:1304-1310. (**Exhibit 5**).

6. Deeths, M.J., et al. (1999) "CD8+ T cells become nonresponsive (anergic) following activation in the presence of costimulation". *J Immunol.* 163:102-110. (**Exhibit 6**).

7. Pantaleo, G., Demarest, J.F., Soudeyns, H., et al. (1994) "Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV." *Nature* 370(6489):463-467. (**Exhibit 7**).

8. Pennesi, P., Liu, Z., Ciubotariu, R., Colovai, A., Cortesini, R., and N. Suciu-Foca. (1999) "TCR repertoire of suppressor $CD8^{+}CD28^{-}$ T cell population". *Human Immunol.* 60:291-304. (**Exhibit 8**).

9. Wells, A.D., Gudmundsdottir, H., and L.A. Turka. (1997) "Following the fate of individual T cells throughout activation and clonal expansion. Signals from T cell receptor and CD28 differentially regulate the induction

and duration of a proliferative response". *J. Clin. Invest.* 100(12):3173-3183. (**Exhibit 9**).

Each of the above-listed publications is listed again on the accompanying PTO Form-1449 (**Exhibit G**). Applicants request that the Examiner make the above-listed publications of record in the subject application.

Summary

In view of the remarks made herein, applicants maintain that the claims pending in this application are in condition for allowance. Accordingly, allowance is respectfully requested.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee is deemed necessary in connection with the filing of this Communication. However, if any such fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:

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6/11/03
Alan J. Morrison Date
Reg. No. 37,399

MARKED-UP VERSION OF CLAIMS

38. (Amended) A method of generating a tolerogenic antigen presenting cell (APC) which comprises:

a) contacting [the] an APC with a CD8+CD28- Ts; and

b) causing overexpression, in the APC of step (a), [overexpressing] of mRNA which encodes an inhibitory monocyte inhibitory receptor (MIR), thereby generating a tolerogenic antigen presenting cell (APC).

39. (Amended) The method of claim 38, wherein the inhibitory [monocyte inhibitory receptor] (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

70. (New) The method of claim 38, wherein the CD8+CD28- Ts cells do not express the BY55 marker.

MARKED-UP VERSION OF SPECIFICATION

Page 4, line 32 through page 5, line 26:

Understanding the mechanism which underlies the induction of immunologic tolerance is crucial to the development of strategies for treatment of auto-immune diseases and allograft rejection. Although the concept that T suppressor cells (Ts) downregulate the immune response has long been accepted, the existence of a distinct population ~~of~~ lymphocytes that mediates suppression has not been convincingly demonstrated. In previous studies, human T cell lines (TCLs) were utilized to analyze the suppressive effects of $CD8^+$ $CD28^-$ T cells in allogeneic, peptide specific and xeno-specific responses. In each case, $CD8^+$ $CD28^-$ T cells inhibit proliferation of $CD4^+$ T helper lymphocytes (Th) with cognate antigen specificity. These $CD8^+$ $CD28^-$ T cells display the critical functional characteristics of T suppressor cells. Similar to the induction of $CD8^+$ cytotoxic T cells (Tc) by Th, this process depends on antigen presenting cells (APC) acting as a "bridge" between MHC-class I specific $CD8^+$ and class II specific $CD4^+$ T cells. A possible explanation of Ts-mediated suppression is their ability to modulate the function of APCs. The fourth series of studies herein show that $CD8^+CD28^-$ Ts directly inhibit the CD40 signaling pathway of APC by a contact-dependent mechanism that renders bridging APCs incapable of inducing $CD4^+$ [The] <u>Th</u> activation. The effects of Ts on the functional state of APC supports the concept that the order in which Ts and [The] <u>Th</u> cells interact with cognate APCs determines the functional outcome of immune responses.

Page 112, lines 16-21:

A chimeric peptide tat-DR4, comprising residues 49-57 of HIV-1 tat and residues 64-88 of DRB1*0401 was purchased from Chiron Technologies, Australia. The purity of the peptide was >85% as determined by reverse-phase HPLC. The amino acid sequence of this peptide is as follows: RKKRRQRRRQKDLLEQKRAAVDTYCRHNYGVGES (SEQ ID NO:382).

Page 130, line 32 through page 131 line 5:

The aim of the present study was to investigate whether the suppressor effect requires the concomitant interaction between Ts, [The] Th and APCs or sequential two cell interactions (first, between Ts and APCs and next, between ''suppressed'' APCs and [The] Th) and whether it is mediated by inhibition of the CD40-signaling pathway.

Page 132, line 20:

Suppression of CD40L Expression on Activated [The] Th Cells

Page 134 line 19 to page 135 line 5:

It is possible that Ts act directly on Th, inhibiting the expression of CD40L or, alternatively, they may act on APCs, blocking the CD40 signaling pathway. To discriminate between these two possibilities, first determined was whether Ts can inhibit Th in the absence of APCs. Experiments in which allospecific Th and Ts were co-cultured in the presence of mAb anti-CD3 showed that Ts do not inhibit Th proliferation or CD40L expression (Figs. 21A, 21B). In contrast, when allospecific Th and Ts are cultured together with the APCs used for priming, both the expression of CD40L and the proliferative capacity of Th are inhibited (Figs. 21C, 21D). These results indicate that the suppressive activity of Ts on Th [proliferation] proliferation is not determined by the direct interaction between Ts and Th and that it requires

the [presence] presence of APCs. This finding is consistent with the previous observation that Ts and Th must recognize the same APC for suppression to occur [5, 6]. It is, therefore, possible that whether APCs can or cannot activate Th depends on their previous encounter with either $CD4^+$ Th or $CD8^+CD28^-$Ts.

Page 140, line 28 through page 141, line 11:

The data herein support a model in which T-cell mediated suppression can result form the sequential interaction between first, [TS] Ts and APCs and next, ''suppressed'' APCs and Th (Fig. 25). In this regard the present findings confirm and extend the ''temporal bridging'' model recently described to account for the complex role that APCs play in Th-mediated generation of $CD8^+$ Tc[2-4]. Furthermore, the present data complement the finding that CD40 signaling is essential for conditioning APCs, by demonstrating that Ts inhibit this pathway. New data show that Ts inhibit [The] Th-induced activation of [NF-B] NF-kB in APC, thus interfering with the upregulation of B7 costimulatory molecules (Li, J., Liu, Z., Jiang, S., Cortesini, R., Lederman, S., Suciu-Foca, N. submitted).

Page 146, lines 2-29:

In the first through fourth series of experiments, we identified and characterized human antigen specific T suppressor cells (Ts). It was shown that Ts inhibits the costimulatory activity of APC blocking NF-κB activation and transcription of costimulatory molecules. To explore the underlying mechanism we used for allostimulating peripheral blood B cells or cells from the dendritic cell line KG-1. Total RNA prepared from KG-1 or from B cells that have been exposed to allospecific Th, Ts [of Th/Tz] or Th/Ts mixtures for 12 hours was used in a cDNA micro-array system to identify genes which are differentially expressed in APC. Although transcription of a wide array of genes was suppressed, expression of 10-15 genes was up-regulated >2-3

fold in APC cocultured for 12 hours with Ts or Ts/Th mixtures. Included in this latter group are the Monocyte Inhibitory Receptor (MIR-10 or ILT4), ILT2 (MIR7), and ILT3. MIR-10, MIR7 (ILT2) and ILT3 belong to a family of leukocyte inhibitory receptors (LIRs) which bear homology to killer inhibitory receptors (KIRs). These molecules interact with MHC-class I molecules via Ig-like domains and regulate negatively the activation of APC, recruiting an inhibitory signaling molecule, [thyrosine] tyrosine phosphatase SHP-1. These data indicate that Ts-induced suppression of APC is based on an active mechanism by up-regulating the expression of a class of inhibitory receptors which transmit negative inhibitory signals in APC. Ts provides an essential regulatory mechanism through which immune tolerance can be achieved.